

Chlamydia trachomatis serovars in 56 PCR-positive clinical specimens

Number of inclusions per coverslip	Number of samples of serovar									
	B group			Intermediate		C group				
	Bb*	E	D	F	G	Ca*	H	Ia	J	K
0-10	4	13	1	15	3	1	4	2	2	1
10-25	0	2	3	2	0	0	0	0	1	2
Total	4	15	4	17	3	1	4	2	3	3

*urogenital Ba and C isolates are termed Bb and Ca respectively.⁶

Diagnostic Systems was employed in order to use residual sample intended for culture. Despite this tenfold dilution and the deliberate use of clinical specimens with few inclusions in culture, all culture-positive samples were also positive by Amplicor PCR. False-negative PCR results in other studies were possibly the result of inhibitors in the sample. In the present study, the further dilution of the clinical sample has apparently reduced the impact of inhibitors without compromising the sensitivity of the procedure.

Although theoretically one *C trachomatis* elementary body may give rise to an inclusion in culture, it would not be surprising that the elementary body to inclusion-forming unit (IFU) ratio of *C trachomatis* in clinical specimens would be similar to the particle to plaque-forming-unit ratio of mammalian viruses (that is, 100 to 1000) as they both must engineer invasion of an eukaryotic cell and takeover of the cellular machinery while both are subject to inactivation during transport. As there are at least 10 copies of the plasmid per chlamydial elementary body, the ratio of plasmid PCR targets to IFU is then probably superior to 1000. This might explain why a tenfold dilution of the clinical sample did not decrease the sensitivity of Amplicor PCR.

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- 1 Quinn TC. Recent advances in diagnosis of sexually transmitted diseases. *Sex Transm Dis* 1994;21:S19-S27.
- 2 Mahony JB, Luinstra KE, Jang D, Sellors J, Chernesky MA. *Chlamydia trachomatis* confirmatory testing of PCR-positive genitourinary specimens using a second set of plasmid primers. *Mol Cell Probes* 1992;6:381-8.
- 3 Loeffelholz MJ, Lewinski CA, Silver SR, et al. Detection of *Chlamydia trachomatis* in endocervical specimens by polymerase chain reaction. *J Clin Microbiol* 1992;30:2847-51.
- 4 Hipp SS, Han Y, Murphy D. Assessment of enzyme immunoassay and immunofluorescence tests for detection of *Chlamydia trachomatis*. *J Clin Microbiol* 1987;25:1938-43.
- 5 Frost EH, Deslandes S, Bourgaux-Ramoisy D. *Chlamydia trachomatis* serovars in 435 urogenital specimens typed by restriction endonuclease analysis of amplified DNA. *J Infect Dis* 1993;168:497-501.

- 6 Frost EH, Deslandes S, Gendron D, Bourgaux-Ramoisy D, Bourgaux P. Variation outside variable segments of the major outer membrane protein distinguishes *Chlamydia trachomatis* from urogenital isolates of the same serovar of *Chlamydia trachomatis*. *Genitourin Med* 1995;71:18-23.

Urethral flora in adolescent boys

Since the information about the composition of the normal urethral flora in sexually inactive young males is limited, it is difficult to know exactly the significance of various isolates from men with urethritis. After the commencement of sexual activity normal urethral flora is bound to alter, we undertook the study of sexually unexposed individuals. The study was undertaken (1) to ascertain the normal flora of the anterior urethra in 50 adolescent boys (aged 13 to 17 years) before commencement of sexual activity (Group A), (2) to evaluate the flora of 50 recently married men in monogamous relationship with no history of sexually transmitted diseases (STDs) (Group B) and (3) to identify various pathogens based on their isolation in 50 patients with non-gonococcal urethritis (NGU) (Group C) and 50 non-urethritis STD patients (Group D) and comparing them with the isolates from the sexually unexposed adolescents. Use of systemic antibiotics in the preceding two weeks was the criterion for exclusion.

If the secretions were scanty milking of urethra was done prior to the collection of samples after holding urine for at least 4 hours. Gram staining of the urethral smears was done for pus cells and microorganisms, saline smear was prepared for *Trichomonas vaginalis*, Giemsa staining for giant cells suggestive of herpes simplex virus (HSV) infection and 10% KOH smear for detection of yeasts were also carried out.

Urethral swabs were processed for culture of mycoplasmas, *T vaginalis*, aerobes and anaerobes including *Gardnerella vaginalis* and for yeasts. After obtaining the urethral swabs, 10 ml of first voided urine was collected from each subject for smear and culture of *T vaginalis*. For culture, 10 ml of mid-stream urine was collected. 5 ml venous blood was drawn for VDRL and HIV serology (ELISA). Data were analysed by using the chi square test (with or without Yate's correction). Data from Group A were compared separately with data from Groups B, C and D.

All smears were negative for *T vaginalis* and giant cells. KOH smear for yeasts were positive in 2 (4%) NGU cases. None of the urine specimens on culture contained more than 10⁴ bacteria/ml, the cut off count for significance. VDRL test and HIV serology (ELISA) was negative in all subjects. Different organisms isolated are shown in the table.

Sexually unexposed adolescents had predominantly the aerobic flora which mostly included the resident cutaneous flora viz

Isolation of different organisms from urethra of adolescent boys and other groups

Organisms	Number of cases (%)				p values		
	Adolescents n = 50 (Group A)	Young males (monogamous relationship) n = 50 (Group B)	NGU n = 50 (Group C)	Other STDs n = 50 (Group D)	A vs B	A vs C	A vs D
<i>Ureaplasma urealyticum</i>	2 (4%)	19 (38%)	26 (52%)	17 (34%)	< 0.01	< 0.01	< 0.01
<i>Mycoplasma hominis</i>	1 (2%)	1 (2%)	3 (6%)	2 (4%)	—	NS	NS
<i>Mycoplasma genitalium</i>	0 (0%)	0 (0%)	0 (0%)	0 (0%)	—	—	—
<i>Trichomonas vaginalis</i>	0 (0%)	0 (0%)	1 (2%)	0 (0%)	—	—	—
Aerobes	33 (66%)	15 (30%)	30 (60%)	35 (70%)	< 0.01	NS	NS
<i>Staphylococcus aureus</i>	1 (2%)	3 (6%)	8 (16%)	9 (18%)	NS	< 0.05	< 0.05
<i>Staphylococcus epidermidis</i>	14 (28%)	3 (6%)	7 (14%)	10 (20%)	< 0.01	NS	NS
Diphtheroids	10 (20%)	5 (10%)	10 (20%)	13 (26%)	NS	NS	NS
<i>Corynebacterium xerosis</i>	6 (12%)	3 (6%)	5 (10%)	7 (14%)	NS	NS	NS
<i>Corynebacterium ovis</i>	4 (8%)	2 (4%)	5 (10%)	7 (14%)	NS	NS	NS
<i>Enterococcus faecalis</i>	1 (2%)	0 (0%)	0 (0%)	0 (0%)	—	—	—
<i>Micrococcus</i>	2 (4%)	0 (0%)	0 (0%)	0 (0%)	—	—	—
α -Haemolytic streptococcus	2 (4%)	0 (0%)	0 (0%)	0 (0%)	—	—	—
<i>Lactobacillus</i>	3 (6%)	0 (0%)	0 (0%)	0 (0%)	—	—	—
<i>Gardnerella vaginalis</i>	1 (2%)	0 (0%)	2 (4%)	0 (0%)	—	NS	—
<i>Acinetobacter</i>	0 (0%)	2 (4%)	0 (0%)	0 (0%)	—	—	—
Anaerobes	—	—	—	—	—	—	—
<i>Peptostreptococcus</i>	0 (0%)	6 (12%)	7 (14%)	8 (16%)	—	—	—
<i>Candida albicans</i>	0 (0%)	0 (0%)	2 (4%)	0 (0%)	—	—	—
Mixed growth	4 (8%)	10 (20%)	20 (40%)	16 (32%)	< 0.05	< 0.01	< 0.01
Bacterial growth of no significance	2 (4%)	2 (4%)	1 (2%)	1 (2%)	NS	NS	NS
Sterile (No growth)	15 (30%)	14 (28%)	11 (22%)	12 (24%)	NS	NS	NS

p < 0.01 and p < 0.05 = Significant.
NS = Not significant (p > 0.05).

Staphylococcus epidermidis and *Lactobacillus* etc and hardly any potential pathogens. *Lactobacilli*, *Haemophilus vaginalis* and α -haemolytic streptococci have been reported as part of the urethral flora in men without urethritis.¹ *Ureaplasma urealyticum* known to be present in the urethra of asymptomatic men,² was found in a significantly small number in adolescents as compared with all the other three groups (p < 0.01). In the sexually active men the picture was more clear. Aerobes did constitute major flora in all the groups but the incidence of *Staphylococcus aureus* was significantly more in the NGU and the non-urethritis STD group compared with men in monogamous relationship and unexposed adolescents. The role of *S. aureus* in the causation of NGU has been suggested by some workers.³ The resident flora like *S. epidermidis* was replaced by other organisms to varying degrees in men who were sexually active. *G. vaginalis* has been isolated from only few of the men with urethritis and is considered as a commensal which may rarely acquire pathogenicity.^{4,5} Peptostreptococci were isolated only from the urethra of sexually active men and their presence in a significant number of men deserves attention. Significantly more men in the NGU and STD groups had mixed growth of organisms compared with the sexually unexposed group where the isolates were obtained more often in pure growth. Subsequent to the sexual encounter the vaginal flora (normal or altered) contributes to the number and species of urethral isolates. Presumably these newly acquired organisms have a primary or contributory role in the causation of disease.

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- 1 Bowie WR, Pollock HM, Forsyth PS, et al. Bacteriology of urethra in normal men and men with non-gonococcal urethritis. *J Clin Microbiol* 1977;6:482-8.
- 2 Altucci A, Varona GL, Catalano G, Manguse L. Mycoplasmas in human genitourinary pathology. *Path Microbiol* 1971;37:89-91.
- 3 Mehta VS, Rana VS, Vaishnav VP. A bacteriological study of acute urethritis. *Ind J Pathol Bacteriol* 1967;10:170-6.
- 4 Kumar B, Sharma M. Carriage of *Gardnerella vaginalis* in the urethra of Indian men. *Indian J Med Res* 1994;99:252-4.
- 5 Lefevre JC, Lepagneur JP, Bauriand R, Bertand MA, Blauc C. Clinical and microbiologic features of urethritis in men in Toulouse, France. *Sex Trans Dis* 1991;18:76-9.

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Screening for asymptomatic *Chlamydia trachomatis* infection in male students by examination of first catch urine

Chlamydia trachomatis causes non gonococcal urethritis and epididymitis in men and pelvic inflammatory disease in women and its role in tubal factor infertility has been well defined. Infection with *C. trachomatis* can also be asymptomatic; about 4%¹ of men attending genitourinary medicine clinics who are infected with *C. trachomatis* are asymptomatic compared with approximately 70% of infected women attenders.² The incidence of *C. trachomatis* in genitourinary clinic female populations is in the range of 5-17.6% and recent publications have shown a decrease in incidence in this group.³ However Taylor-